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1: J Neurochem. 1999 Nov;73(5):1859-70.

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Isolation of a potential neural stem cell line from the internal capsule of an adult transgenic rat brain.

Kilty IC, Barraclough R, Schmidt G, Rudland PS.

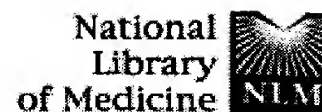
Cancer and Polio Research Fund Laboratories, School of Biological Sciences, University of Liverpool, England.

A thermosensitive mutation of simian virus 40 large T antigen (LTA) gene, the tsA58 gene, was cloned downstream of the 6-kbp neurofilament light chain promoter in pPOLYIII and injected into the pronucleus of fertilised oocytes of Sprague-Dawley rats to develop a strain harbouring six copies of the transgene. Immunocytochemical staining of hemizygous adult tissues with antibodies to the C-terminus of LTA showed that the inactive form of LTA was expressed only in the fibres of the internal capsule and in the choroid plexus of the brain. Culturing the former region at 33 degrees C, the permissive temperature for LTA, yielded a cell line, NF2C, which produced active LTA and grew at 33 degrees C but which produced only inactive LTA and eventually died at the non-permissive temperature of 39 degrees C. This clonal cell line was heterogeneous at 33 degrees C, producing the precursor neuronal cell marker nestin and the glial-specific markers glial fibrillary acidic protein, vimentin and S100A1, as well as weakly producing the neuronal cell markers 68-kDa neurofilament protein (NF68) and microtubule-associated protein 2 (MAP2) in different subpopulations of cells. However, at 39 degrees C, the cells produced dendritic, neuronal-like processes and elevated levels of NF68 and MAP2, as well as the neuronal markers synaptophysin, neurone-specific enolase, and low levels of tau, all determined by western blotting and immunofluorescent staining. Basic fibroblast growth factor enhanced the growth of the cells at 33 degrees C but also enhanced the formation of dendritic neuronal-like processes at 39 degrees C. It is suggested that NF2C represents a potential stem cell line from adult brain that expresses precursor and glial cell markers at 33 degrees C but undergoes partial differentiation to a neuronal cell phenotype at 39 degrees C.

PMID: 10537044 [PubMed - indexed for MEDLINE]

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Establishment of tsA58 transgenic rats as a source of conditionally immortalized cell lines with differentiated functions.

Takahashi R.

YS New Technology Institute Inc., 519 Shimoishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi 329-0512, Japan.

To isolate a variety of rat cell lines with differentiated functions, we developed transgenic rat lines that ubiquitously express the temperature-sensitive large T-antigen gene of the simian virus 40 (SV40) tsA58 mutant under the control of the SV40 large T-antigen promoter. These rats might be advantageous for simultaneously establishing cell lines from different tissues of rats with the same genetic origin. The transgenic rat lines transmit a functional copy of the transgene and were bred with sib mating to generate the homozygous transgene. The established cell lines from this transgenic rat had temperature dependent growth and retained some of the differentiated functions of each particular tissue, and were useful as a ready source of novel conditionally immortalized cell lines. The possible use and perspectives of these transgenic cell lines are discussed.

PMID: 12012717 [PubMed - indexed for MEDLINE]

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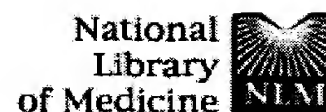
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1: Exp Anim. 1999 Oct;48(4):255-61.

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Establishment of SV40-tsA58 transgenic rats as a source of conditionally immortalized cell lines.

Takahashi R, Hirabayashi M, Yanai N, Obinata M, Ueda M.

YS New Technology Institute Inc., Tochigi, Japan.

To isolate a variety of rat cell lines with differentiated functions, we established transgenic rat lines expressing the temperature-sensitive large T-antigen of simian virus 40 (SV40) tsA58 mutant under the control of the SV40 large T-antigen itself. We microinjected the DNA into 564 eggs of Wistar rat and 23 independent transgenic candidates were obtained. Ten pups died before weaning and eight transgenic rats could not transmit the transgene to the progeny. Finally, five lines of the transgenic rat were established. Although one line (#1511-6) had low reproductivity, the other four lines reproduced normally. Three out of the four lines (#1507-2, #1509-7, #1519-8) appeared normal but the other line had tumors in the brain and subcutaneous tissue at 3 weeks of age (#1511-6), and in the kidneys and subcutaneous tissue at 18 to 19-weeks of age (#1507-5). Fibroblast cells prepared from transgenic fetuses of lines #1507-5 and #1519-8 expressed the transgene and exhibited temperature-dependent growth. Both of the lines (#1507-5 and #1519-8) were successfully generated to be homozygous by sibling mating of transgenic offspring. These transgenic rat lines have bred through many generations and have been established to be a ready source of novel conditionally immortalized cell lines.

PMID: 10591005 [PubMed - indexed for MEDLINE]

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